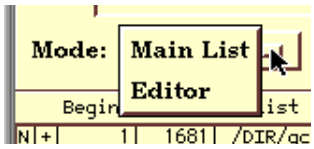
Electropherograms in SeqLab.

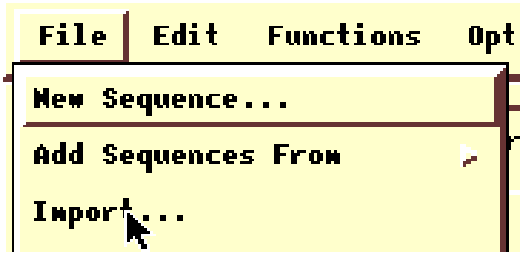
Electropherogram Files can be imported into the editor mode. The editor will display the sequence, while the "traces" can be viewed in a separate window. Base selection with the mouse is coordinated between the two windows. Changes may be done in the Editor window. The trace window shows the original base call and the edited bases. Results of editing can be saved in an RSF file. A pointer in the RSF file associates the original file with the edited sequences so they can be displayed whenever those sequences are in the editor.

There is now a system in place where the DNA Sequencing Core Facility can place the electropherogram files directly in a special directory that only you can access. This tutorial will tell you how to access that directory.

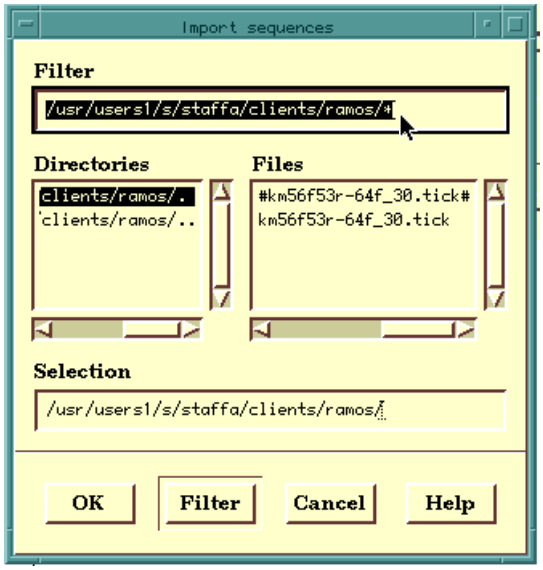
1. Starting from the Main List Mode, make sure nothing is selected in the Main List Window. Hold down the control key and click on anything that is selected in order to de-select it. Change **Mode** to **Editor**



2. Click on the **File** menu and select **Import....**

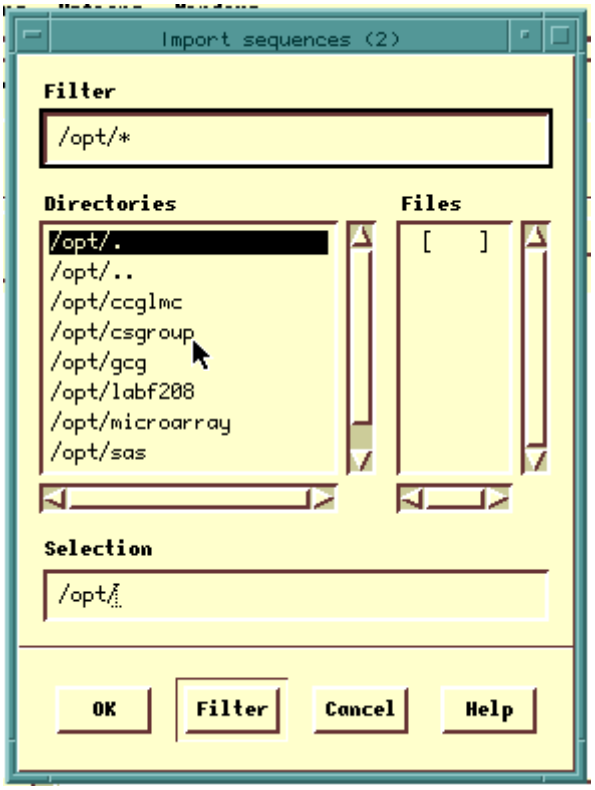


3. In the **Import Sequences** window you must completely change the **Filter** specification. These instructions will minimize typing. Select everything in the Filter text box and delete it with your delete or backspace (on PC) key.



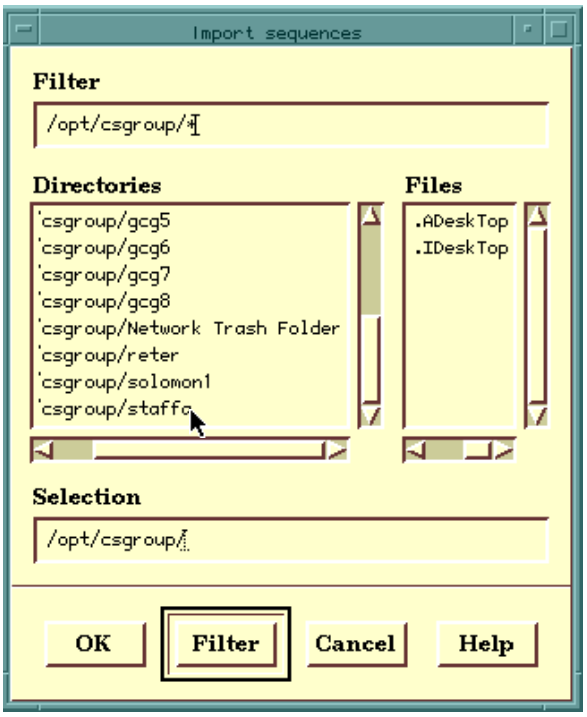
4. In the Filter box type **/opt/*** and press **Return.**

5. When the **Directories** display changes, Click on **"/opt/csgroup"** and click **Filter**. (You may also double click "**/opt/csgroup**")

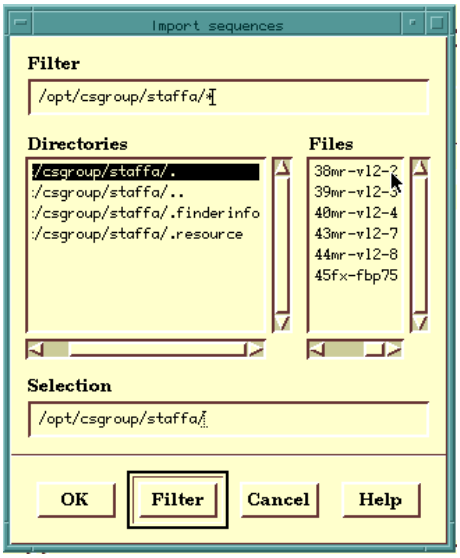


6. In the new Directories display, find and select the directory with **your username**.

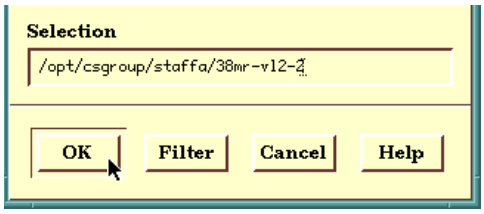
7. Click **Filter** (or press return or double click).



8. When the display changes, click on one of the files in the **Files** panel.

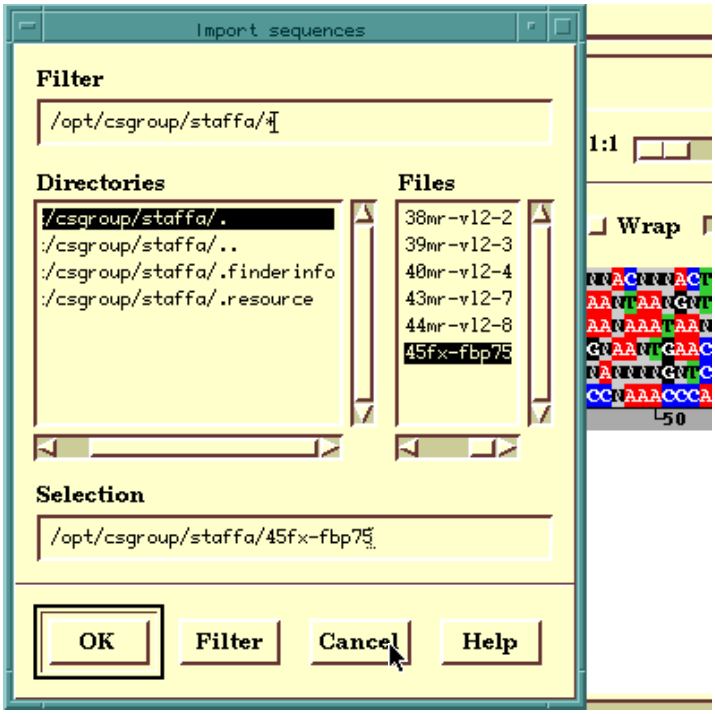


9. Click **OK** to add the selection to the editor.



10. To add several sequences, repeat steps 8. and 9. to add each one.

11. Click **Cancel** when you are done.



Using Electropherograms in the Editor

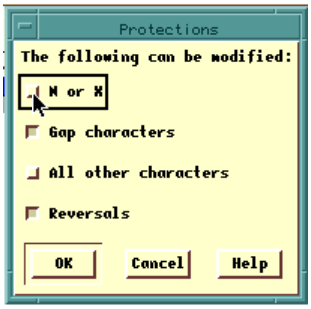
The editor can be used to get your fragments into shape before entering them into GCG's Fragment Assembly System (FAS). The purpose is to remove sufficient ambiguities from the end of the fragments so that contig assembly program, GelMerge, can find the proper overlaps. This may require correcting base calls, inserting or deleting called bases, or even chopping ambiguous ends off the fragment. When a fragment is entered into the Fragment Assembly System (FAS) with GelEnter, it can only be edited with GelAssemble, since the FAS keeps its own copies of the sequences. Although you may later look at the original electropherograms with SeqLab to make decisions about ambiguities in your contigs, further changes made with SeqLab Editor will not be seen in the FAS (unless you "erase" the fragment in GelAssemble and re-enter the fragment edited in SeqLab), and changes made in GelAssemble will not be seen in SeqLab.

1. Click on the Sequence Name to select it.

2. So you can change the called bases, change the editing mode to **Overstrike.** (If you need to insert bases, you will need to go back to **Insert.**)

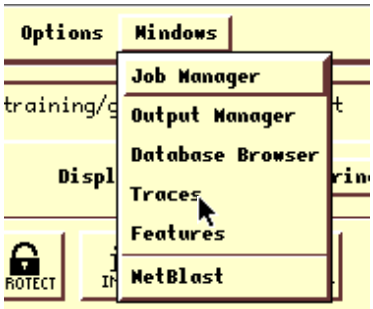
3. Click on the lock icon to see protections.

4. Change the protections to allow just the changes you will need.

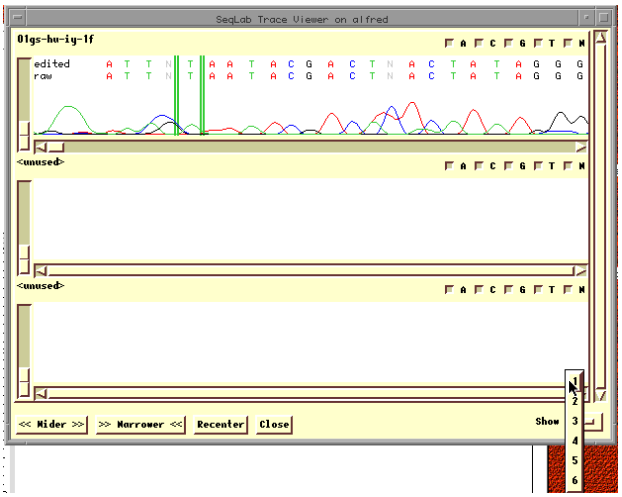


5. Click **OK.**

6. Click on the **Windows** menu and select **Traces**.

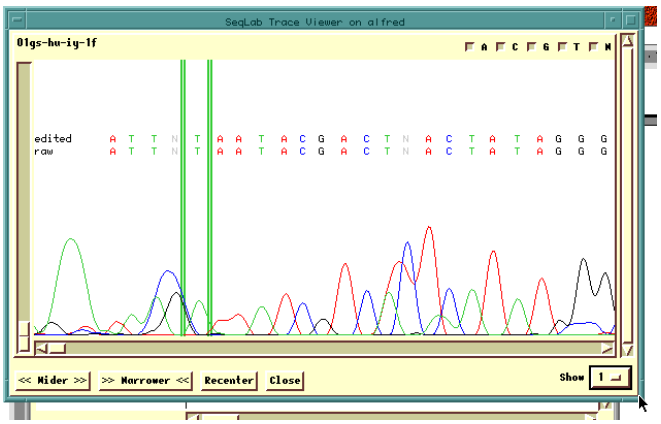


7. A three-paneled window will appear. In the bottom right, change **Show** from **3** to **1.**

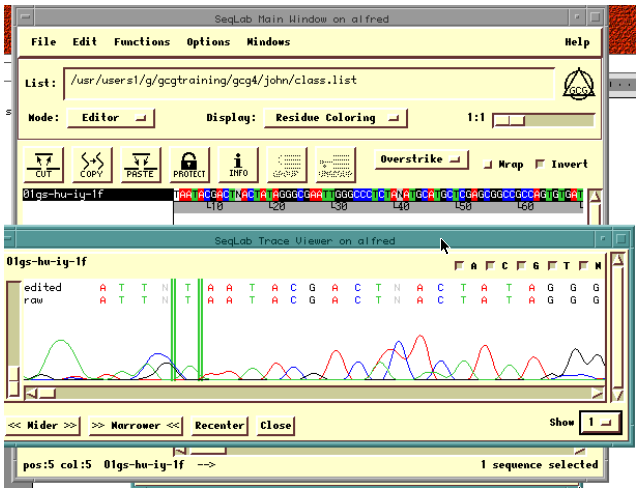


8. The following instructions are intended to facilitate viewing both the trace and the sequence as displayed in the editor, and to switch between them.

9. With your mouse, grab the lower right-hand corner of the Motif window and drag it upward till the window is less than half the height of your screen.

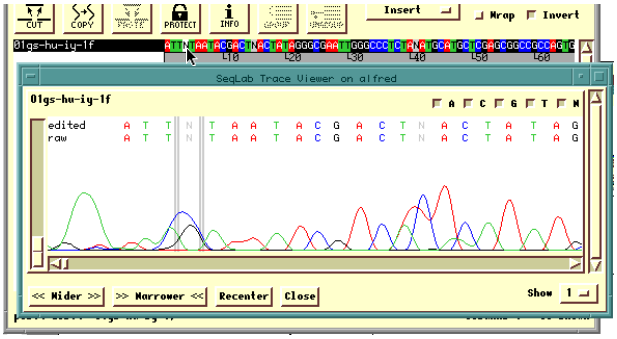


10. Grab the title bar and put the Trace Viewer below the sequence in the editor so that both the trace and the sequence are visible.



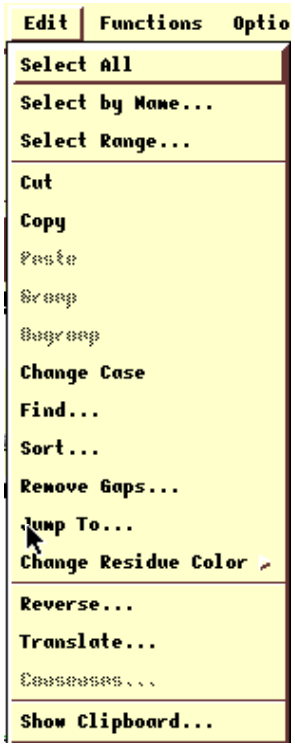
Note: Even ambiguity symbols can help the GelMerge program find overlaps if you can narrow down your choices. IUB/GCG symbols can be found by Clicking **Help** in the upper right hand corner, selecting **On the Wisconsin Package,** and scrolling down in the Subtopics panel till you see **Appendices**. Choose this and press Return. **Choose Appendix III** and press return. Scroll down in the upper window to see the table.

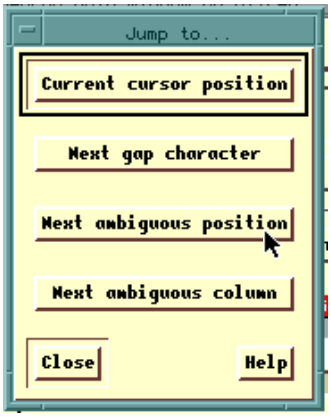
11. Click on the first “N” in the trace. Use the slide on the left to increase the scale of the trace till you can discern perhaps what base you would like the”N” to be. The IUB code for C or G is S.



12. Click on the N in the Editor window, which should come forward, and type an “s”.

13. In the editor window, click on the Edit menu and select **Jump To**... Click on **Next ambiguous position**. The editor’s cursor will jump to that position, but you must then click on that position in the Editor to get the Trace view to follow.

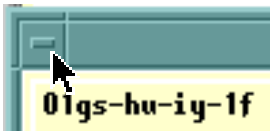




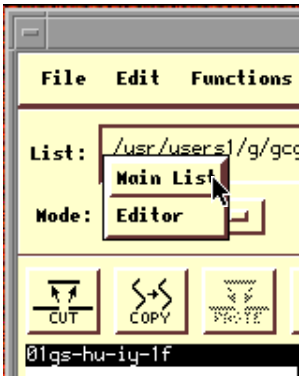
14. Use the slider on the right of the Trace View to change the height of the traces. Go to the editor when you are ready to make a base call.

15. Repeat this operation until you have fixed-up your sequence to your satisfaction.

16. Close the trace window by clicking the button in the upper left.



17. Change mode from Editor to Main List



18. Save the resultant RSF file. You may edit the name to make it more meaningful, but preserve the ".rsf", please. A link to the electropherogram will also be saved in this file for future reference.